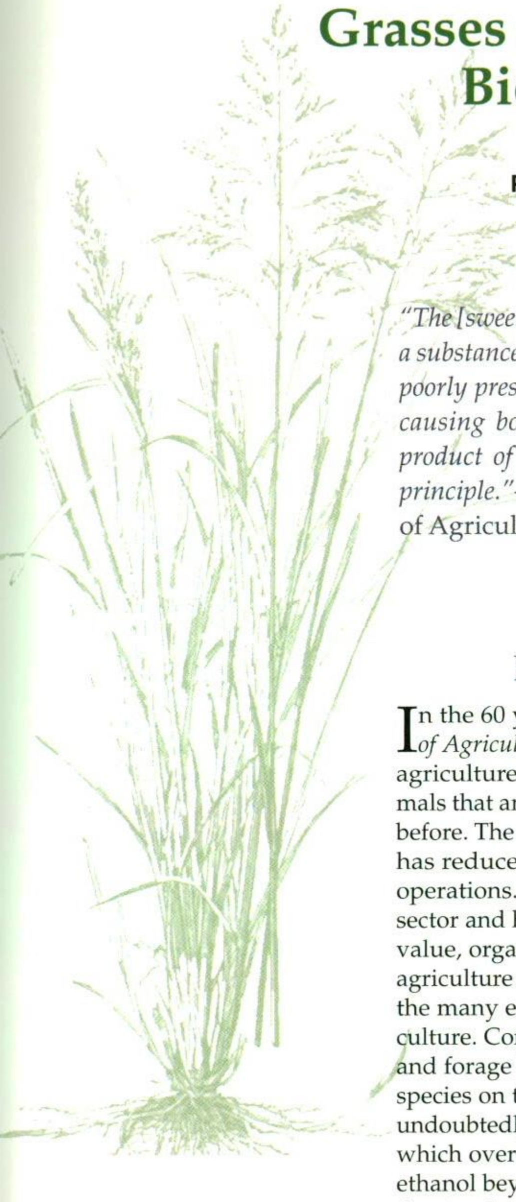


Grasses and Legumes for Bio-based Products 13

Paul J. Weimer and J. Bradley Morris

"The [sweetclover] species grown in this country contain coumarin, a substance that has a vanillalike odor. Spoiled sweetclover hay and poorly preserved sweetclover silage is frequently toxic to animals, causing both external and internal bleeding. A decomposition product of the coumarin occurring during spoilage is the toxic principle."—R. McKee, in Stefferud, Grass: The 1948 Yearbook of Agriculture, *p. 718*

HISTORICAL PERSPECTIVE

In the 60 years since the publication of *Grass: The 1948 Yearbook of Agriculture* (Stefferud, 1948), much has changed in American agriculture. Advances in livestock genetics have resulted in animals that are larger, grow faster, and are more productive than ever before. The use of high-energy diets, based largely on cereal grains, has reduced the overall demand for forage in confined animal operations. Grazing remains a foundation for the beef and sheep sector and has gained a niche market for dairy, especially for high-value, organic production systems. Yet the emphasis on intensive agriculture and high productivity has diverted the public eye from the many environmental and social benefits of forage-based agriculture. Consequently, development of alternative uses for grasses and forage legumes is essential for long-term efforts to keep these species on the landscape. A major boost for forage production will undoubtedly be provided by the nascent cellulosic ethanol industry, which over the coming decades seeks to expand the production of ethanol beyond the approaching limits to sustainable ethanol production from corn (*Zea mays* L.) and other cereal grains.

This chapter summarizes the current state of the art in identifying and deriving added value from grasses and legumes beyond their traditional primary use as feeds for livestock. Topics considered here are the use of grasses and forage legumes as structural materials; sources for textile fibers, pulp and paper, commodity chemicals, and specialty chemicals, including enzymes; nutraceuticals; functional foods, and medicinal foods. Not considered in this chapter are certain other "nonforage" uses of grasses and legumes, including the direct use of live plants in conservation plantings, turf, and ornamental gardening, or in phytoremediation of soil and water contamination. Also not considered here are the use of grasses and legumes as feedstocks for energy production via combustion, pyrolysis, or fermentation (the central focus of Chapter 12 [Casler et al., 2009, this volume]), although the fermentation of grasses and legumes to other commodity and specialty chemicals is discussed here. For many of the commercial or potential products described, a residual fraction can be produced that may retain certain traditional uses, such as for animal feed. Implicit in the discussions below is that the practicality of using grasses and legumes for various bioproducts will depend on changes in the face of U.S. agriculture, particularly with regard to the use of both forages and grain crops for biofuels production. The projected massive growth of the biofuels industry may divert cellulosic crops away from uses both historical (livestock feed) and new (nonfuel bioproducts), or alternatively, it may stimulate a renaissance of grassland agriculture that will increase the contribution of forages to biofuels, bioproducts, and livestock feed alike.

STRUCTURAL USES

Structural uses of plants include both their inclusion in engineered composite materials and their direct use in building construction. In the former case, natural fibers such as kenaf (*Hibiscus cannabinus* L.), flax (*Linum usitatissimum* L.), and hemp [*Cannabis sativa* ssp. *indica* (Lam.) E. Small & Cronquist] have been shown to be useful as reinforcing agents for "green" composite materials, and more recent work has suggested that agricultural residues (corn stalks, rice straw) and other grasses have similar capabilities. Studies have shown that a formulation consisting of Indiangrass (*Sorghastrum nutans*), extruded soy [*Glycine max* (L.) Merr.] flour, and polyester amide can be injection molded to produce various high-strength composite materials. Performance of the materials can potentially be enhanced by pretreating the grass with alkali (lye and other basic solutions) to remove the hemicellulose and lignin, two natural polymers present in plant cell walls that interfere with the interaction between the additive and cellulose fibers, which are the chief source of tensile strength in grasses.

Potential use of plants in engineered composites extends beyond conventional crops. A cane-like, sterile perennial hybrid often referred to as E-grass or elephant grass (*Miscanthus × giganteus*) is vegetatively propagated and may reach a height of 10 feet (3 m). This plant is the subject of ongoing work to include its fibers as reinforcing agents to strengthen biodegradable plastics in a variety of products including fiberboard and auto body parts.

A second use of grasses and legumes is as building construction materials. Grasses in particular have physical properties that impart strength and insulation sufficient to serve as the basis for a whole new (or at least newly discovered) type of building construction.

At the extreme end of grass-based construction materials is bamboo, a woody perennial evergreen that is actually a true grass (family Poaceae). Taxonomically, the bamboo subfamily contains about a thousand species distributed into 91 different genera, with a wide distribution in tropical and subtropical areas, including the subtropical United States. Diversity of properties of the group contributes to bamboo's reputation as the "plant of 5000 uses."

Within the bamboo subfamily are species that are the tallest of all grasses and that have the fastest maximum growth rate of any plant (up to 3.1 feet [94 cm] per day in young shoots). The preferred species for timber include certain representatives of the genera *Dendrocalamus* [*D. asper* (Schult. & Schult. f.) Backer ex K. Heyne, *D. brandisii* (Munro) Kurz, and *D.* spp.] and *Gigantochloa* [*G. apus* (Schult. & Schult. f.) Kurz ex Munro, *G. atter* (Hassk.) Kurz ex Munro, and *G.* spp.]. Most growth occurs in a spurt of about three months' duration, during which the plant nearly reaches full height (100 feet [30 m] or more) and diameter (up to 1 foot [30 cm]). Subsequent thickening of the "trunk" (stem) occurs over the course of about seven years, allowing harvest of the light, strong wood at a younger age than for most tree species. Most commercial bamboo is plantation grown, and recent production trends include attempts to intercrop the plant with nitrogen–fixing trees for enhanced environmental sustainability. Bamboo is also a pioneer plant well suited to restoring degraded lands and controlling soil erosion, a role enhanced by its complex root system, which acts as a natural barrier to water flow.

Beyond its use as structural lumber, bamboo is used in interior applications (furniture and paneling, along with its increasingly popular use as wood flooring). In addition to its well-known culinary and ornamental uses, bamboo is commonly used in decorative artwork, cutting boards

NO "THREE LITTLE PIGS JOKES" PLEASE!

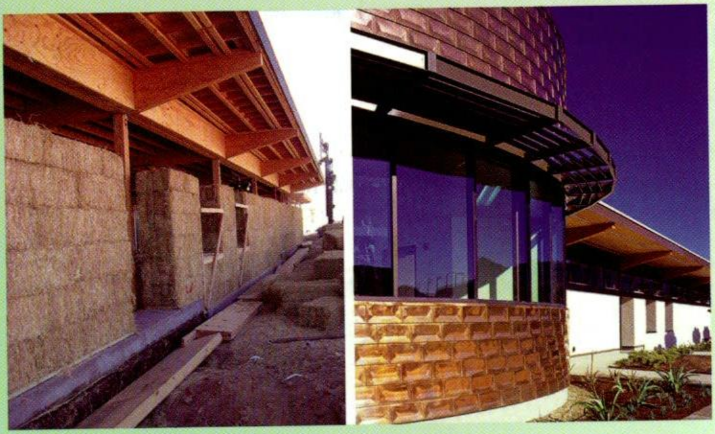
Straw bale home built in Arthur, Nebraska, in 1925. (Source: USDA)

Straw bale construction represents an exciting new trend in building practical and environmentally sound residential homes. Originally built in the 1880s on the treeless Great Plains, straw bale houses proved their worth by keeping settlers comfortable through the region's climatic extremes. More recently, straw bale homes have regained popularity in the United States, particularly in the Southwest, where they have been combined with passive solar design to yield highly energy efficient buildings (USDOE, 1995).

Several construction styles have been developed. The most popular is the "Nebraska style" building, which features tightly packed straw bales as load-bearing walls. Exterior coatings of stucco or adobe, and an interior coating of plaster assure a dry, vermin-free structure that can be rapidly constructed using relatively unskilled labor. These structures are remarkably energy efficient because of the strong insulating properties of straw (R-values typically around 2.4 to 3.0 per inch [per 2.5 cm]) and are surprisingly fire resistant if the walls are properly sealed. Total construction costs vary widely from one-tenth to as much as more than twice that of conventional wood-framed buildings. Greatest potential savings are in areas where lumber is expensive or not readily available.

Nebraska-style straw bale homes typically use standard three-wire bales (approximately 16 by 24 by 45 inches [41 by 61 by 114 cm] and weighing 75 to 85 pounds [34 to 39 kg]) composed of straws from cereal grains (wheat, oat, rye, rice), although in principle, any thick-stemmed grass can be used. The primary requirements of a good straw bale are that the plant material be dry (<14% moisture) and tightly packed to support weight in staggered vertical courses. The courses are reinforced with steel bars or wooden poles. After installation of roof beams and trusses, the structure is allowed to settle, netting is applied to the inside and outside walls, and the exterior and interior coatings are applied.

With the recent emphasis on energy efficiency, natural materials and "green construction," straw bale construction appears positioned to provide an attractive alternative for people looking for novelty and practicality in a new home. Such building trends have even begun to spill over into other construction, including the recently completed U.S. Post Office building in Corrales, NM.



Straw bale construction used in the Santa Clarita Transit Maintenance Facility (Santa Clarita, CA), before and after. (Photo on right by John Edward Linden; photos courtesy of HOK) and kitchen utensils, cages, enclosures for aquaculture, sporting equipment (skateboards, snowboards, surfboard, and fly-fishing rods), and over a dozen musical instruments.

Overall, structural uses for grasses are increasing and are likely to continue to do so in a world in which environmental consciousness and "eco-friendly" products become more mainstream.

PULP AND PAPER

Kenaf is a short-day, annual plant grown for the soft bast fiber (i.e., the fibers of the phloem tissue or the "inner bark") in its stem. There are approximately 200 species of kenaf and related *Hibiscus*, including annuals and perennials, which are widely distributed geographically. Kenaf is closely related to cotton (*Gossypium hirsutum* L.), okra [*Abelmoschus esculentus* (L.) Moench], and hollyhock (*Alcea rosea* L.). Production centers primarily in Africa, Asia, China, India, and more recently in Texas. Since 1960, great interest has arisen for growing and using kenaf for the manufacture of newsprint and other pulp and paper products. Several varieties are in cultivation using standard production practices, including direct seeding with conventional grain drills. During harvest, kenaf plants are topped off at 12 feet (3.7 m) and dried in windrows. Partially dry stalks are removed from the field 10 days later, cut into 1-foot (30-cm) billets, and blown into a trailer. Soon after, kenaf bast and core fibers are separated. The former can be pulped as a vegetable fiber for special pulp and paper uses, or it can be blended with plastic for injection molding purposes, and mats can be processed for use as instant lawns or as blends with textiles. Core fibers are processed into oil absorbents, soil-free potting mixes, animal bedding, packing material, organic filter for plastics, additive for drilling muds, and insulation.

Several species of grasses, including *Miscanthus* × *giganteus*, timothy (*Phleum pretense* L.), big bluestem (*Andropogon gerardii* Vitman), bamboo—and even corn stover—have been used to make paper having properties somewhat different from those of conventional papers derived from wood pulp. However, this use has been restricted almost exclusively to home hobbyists and to specialty shop customers who place a higher value on organic sourcing and other social considerations over direct economic considerations. Such uses are unlikely to represent much of an opportunity for, or a threat to, the mainstream paper industry.

CHEMICALS DERIVED FROM LEGUMES AND GRASSES

Chemicals derived from forages include enzymes, nutraceuticals, functional foods, and medicinal chemicals. Those that have sufficiently high value can sometimes be economically recovered by direct extraction, and the low-value residue discarded. However, economic benefit is more likely if multiple components can be recovered, an approach consistent with the modern concept of a "biorefinery." The underlying strategy of biorefining is to separate components having different uses from one another to produce unique products for different applications. Such fractionation is likely to be more promising for legumes than for grasses, which display relatively minor compositional differences among plant parts. Fractionation should, in principle, be applicable for generation of both natural products from cultivars produced by classical plant breeding and nontraditional products from transgenic crops (i.e., crops containing genes introduced from other life forms). The potential for fractionation has been examined most extensively in alfalfa (*Medicago sativa* L. ssp. *sativa*). Although it typically yields less herbage per acre than some grasses, alfalfa is prized for its ability to fix atmospheric nitrogen (eliminating nitrogen fertilizer costs), its high protein content, and the rapid ruminal fermentation of its fiber. Moreover, as noted below, alfalfa is a potential source for several specialty phytochemicals.

NUTRACEUTICALS OR DIETARY SUPPLEMENTS

Major legumes including soybean and alfalfa contain numerous phytochemicals known as nutraceuticals, or dietary supplements, that are currently on the market. Soybean is the most important legume produced worldwide, and several nutraceuticals, including isoflavones, which act primarily as antioxidants in humans, exist in soybean seeds. Dietary aids for diabetics include soybeans

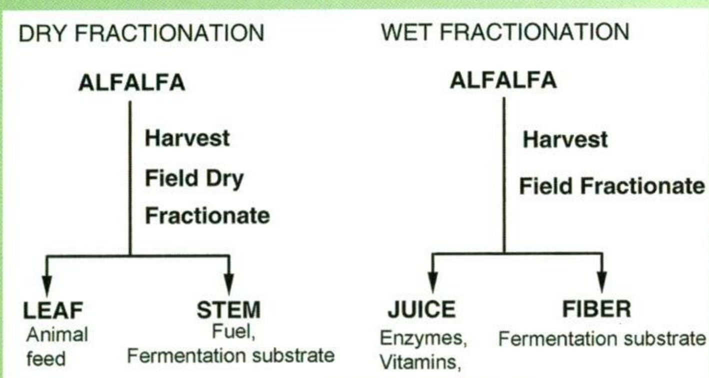
ALFALFA FRACTIONATION: ADDING VALUE FOR FARMERS

Wet-fractionation of alfalfa using a field-scale prototype "juicer." (Photo courtesy of M.C. Boettcher, USDA, Agricultural Research Service)

From both agronomic and product quality standpoints, alfalfa has earned its title as the "Queen of Forages." This nitrogen-fixing, deep-rooting perennial legume is drought and cold tolerant and yields highly palatable, high-protein herbage that makes excellent hay and silage. But alfalfa also has the potential to serve a variety of other uses. Key to these uses is the development of fractionation technologies that can separate the forage into several component streams, each having a unique use.

Two fractionation technologies show promise. *Dry fractionation* involves drying of the cut forage and subsequent separation of the leaf and stem fractions. The leaf meal is a highly digestible source of protein, fiber, and energy for ruminant animals, while the stem fraction, rich in lignin and plant polysaccharides, has a high caloric value for combustion. Dry fractionation nearly reached commercialization in the 1990s as the MinVAP process and may become practical with the recent emphasis on biofuels and their potential role in reducing net greenhouse gas emissions.

Wet fractionation involves squeezing of freshly cut herbage through a press, which yields a juice fraction that contains soluble proteins and numerous phytochemicals. The protein fraction has potential utility both as an animal feed and as a human food additive (Koegel and Straub, 1996). Proteomic characterization has revealed that the wet fractionation process induces proteolytic enzymes and heat shock proteins, resulting in changes in the characteristics of individual proteins in the juice. Beyond these changes in the properties of proteins from conventional cultivars, additional value would potentially accrue from the use of transgenic alfalfa that produces enzymes for various end uses (see main text). For example, phytase, an enzyme which breaks down phytic acid to yield phosphorus that can be absorbed by livestock, has the effect of minimizing excretion of phosphorus, the primary agent of eutrophication of lakes and streams. The other product of wet fractionation, fibrous solids, does not have a defined use at this point, but has been shown in lab-scale experiments to serve as a substrate for fermentations that produce novel wood adhesives (Weimer et al., 2005). While the wet fractionation process requires more processing energy than dry fractionation, it has the advantage of direct and immediate use of the alfalfa without drying, thus obviating the common problem of rain-related reductions in forage quality. Wet fractionation processes are also under development for biorefining grasses.



Simplified schemes for dry- and wet-fractionation of alfalfa for producing different product streams. Dry fractionation performs a separation based on plant anatomy, while the wet fractionation performs a separation based on water solubility of components.

ISOFLAVONOID AND FLAVONOL CONTENT IN GUAR SEEDS

Guar seeds contain additional phytochemicals, including various isoflavonoids and flavones. However, little information regarding variability for such traits is in the literature. The isoflavonoids daidzein and genistein are potential antioxidants, inducers of apoptosis, regulators of host immune systems, and inducers of detoxification enzymes; in addition, they inhibit breast and prostate cancer cells (Messina 1999). Flavonols such as kaempferol and quercetin have been shown to inhibit lung and colon cancer cells as well as protect against ultraviolet radiation (Nguyen et al., 2003; Park et al., 2005).

To test for variation in phytochemical content, guar seeds were ground into a powder using a coffee blender, mixed with 80% methanol, then vortexed briefly and incubated at 80°C. The samples were centrifuged to remove solids, and supernatant was filtered through 0.2-μm syringe filter and injected into a high-performance liquid chromatography system. While significant differences among guar accessions were apparent, the majority of guar accessions produced a much higher amount of kaempferol (ranging from 10.7 to 19.8 mg/100 g) than all other phytochemicals. (Wang and Morris, 2007).

and are marketed as dietary supplements. The phytochemical glycine lunasin from soybean has been shown to be apoptotic as well as chemopreventive. Another component, glycine β–1,3-glucanase, has been shown to have antifungal properties (Polya, 2003). Alfalfa contains saponins, which are known to lower cholesterol. One of the most important phytochemicals found in alfalfa leaves is lutein, a commercially available antioxidant, free radical scavenger, and active agent against age-related macular degeneration (a leading cause of irreversible blindness). Several isoflavones from both soybean and alfalfa are commercially marketed.

Many minor legumes including yellow sweet clover [*Melilotus officinalis* (L.) Lam.], fenugreek (*Trigonella foenum-graecum* L.), subterranean clover (*Trifolium subterraneum* L.), butterfly pea (*Clitoria ternatea* L.), and red clover (*Trifolium pratense* L.) contain possible compounds for use as nutraceuticals. Both guar [*Cyamopsis tetragonoloba* (L.) Taub.] and sunn hemp (*Crotalaria juncea* L.) seed produce variable quantities of dietary fiber, and guar seed produces substantial amounts of flavonols as well. Sweet clover contains the phytochemical coumarin, which is known to decrease pain in humans, and a fenugreek extract lowers blood sugar in diabetics. Subterranean clover contains fisetin, which not only is antibacterial but also has apoptic capability against cancer, while red clover contains the antifungal phytochemical medicarpin and the chemopreventive chemical biochanin. Butterfly pea seed contains the antifungal chemical, clitoria defensin and free radical-scavenging, antioxidant-acting anthocyanin (Polya, 2003). Both Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald] and *Lespedeza capitata* Michx. seeds are being investigated for potential nutraceuticals as well. Wheat (*Triticum aestivum* L.) contains potentially useful phytochemicals with nutraceutical capabilities, such as α and β tocotrienols in wheat germ oil, which act as antioxidants, free radical scavengers, and inhibitors of low-density lipoprotein (LDL, or "bad cholesterol") oxidation. In addition, wheat germ consists of αβγδ-tocopherols, which display antiretinopathy, antioxidant, free radical scavenging, and anti-aging activities. Biochanin, fisetin, β-tocotrienol, and αβγδ-tocopherol are commercially available.

Taken together, grasses and legumes contain a host of compounds that improve the healthfulness and value of foods. This suggests the opportunity not only for growing markets but also for additional research to identify other plants that can serve as nutraceuticals and dietary supplements.

Sunn hemp (*Crotalaria juncea*). (Photo by J. Bradley Morris)

TOTAL DIETARY FIBER IN SUNN HEMP AND GUAR

SUNN HEMP

Sunn hemp is an underutilized legume that is primarily produced in Asia and the valley area of Texas, where its stem fibers are used in the manufacture of twine, cord, and paper. Sunn hemp is also resistant to root-knot nematodes and improves the soil via nitrogen fixation.

Fiber occurs throughout the sunn hemp plant, including the seeds. Total fiber measured as total dietary fiber includes plant nonstarch polysaccharides, oligosaccharides, resistant starch, and lignin. Lignin is found in the water insoluble fraction of fiber, and the polysaccharides can be in the water-soluble or insoluble fraction depending on their structure. Its fiber is used in industry as well as for various purposes related to human health.

In studies of seed composition, seed preparation for fiber analysis was accomplished by grinding sunn hemp seeds in a Spex CertiPrep mixer/mill. After thorough mixing (AOAC Method 991.43; American Association of Official Analytical Chemists, 2000), an enzymatic gravimetric method for total dietary fiber (TDF) was used to analyze fiber. This TDF method includes both soluble and insoluble fractions of fiber in the measurement. Significant variations in TDF were observed among several sunn hemp accessions.

GUAR

Guar is a legume containing galactomannan gum, which is used as a food ingredient and, more recently, as a nutraceutical. Guar originated in India and Pakistan, where it is primarily produced. However, substantial acreage exists in the west Texas area as well. Guar exceeds sunn hemp for fiber usage because much is known about its food additive qualities. Guar seed is 42% endosperm, with mucilage or guar gum predominating. About 80 to 85% of the gum is galactomannan. Guar gum galactomannans are water-dispersible hydrocolloids, which thicken when dissolved in water, leading to their usefulness as food additives for emulsifying, thickening, and stabilization.

Guar seeds were ground in similar fashion as the sunn hemp seeds and mixed. The AOAC Method 991.43 (American Association of Official Analytical Chemists 2000) was used to analyze fiber as well. As with sunn hemp, significant variability for TDF among nine guar accessions was observed.

ETHNOBOTANICAL USES OF LEGUMES AND SELECTED GRASS SPECIES

Ethnobotany is the plant lore of a race or people and the systematic study of such lore.

Some of the ethnobotanical uses shown in this table are based on Native American folklore. Hallucinogenic properties may exist in some species, suggesting possible uses as analogs for or precursors of pharmaceutical research. (Precursors for pharmaceutical research refer to plant substances—in this case, phytochemicals—with potential health-enhancing qualities for pharmaceutical research.)

Species		Country/Native American tribe	Use
Scientific name	Common name		
		Legumes	
Lespedeza capitata	Round head lespedeza	Omaha	Analgesic
Desmanthus illinoensis	Illinois bundleflower	Pawnee	Dermatological aid
Desmodium canadense	Canadian tick trefoil	Iroquois	Gastrointestinal aid
Strophostyles helvola	Wild bean	Iroquois	Dermatological aid
Phaseolus acutifolius	Tepary bean	Papago	Toothache remedy
Tephrosia virginiana	Virginia tephrosia	Cherokee	Dermatological, kidney aid
		Creek	Tuberculosis remedy
Lespedeza cuneata	Sericea	China	Dog, snake bite; dysentery, hernia, toothache, tuberculosis
Lablab purpureus	Hyacinth bean	Iraq	Fever
Indigofera suffruticosa	Anil indigo	U.S. (Hawaii)	Backache
Indigofera tinctoria	Indigo	China	Cancer, dysentery
Desmodium adscendens	Tick clover	Africa	Asthma
		Grasses	
Eriophorum chamissonis	Chamisso's cottongrass	W. Eskimo	Dermatological aid
Bouteloua gracilis	Blue grama	Navaho-Ramah	Dermatological aid
Agropyron repens	Quackgrass	Cherokee	Orthopedic aid
Triticum aestivum	Wheat	Canada	Cancer
Oryza sativa	Rice	Spain	Diarrhea
Hordeum vulgare	Barley	Egypt	Stomach cancer
Zea mays	Corn	India	Pneumonia
Cynodon dactylon	Bermudagrass	U.S.	Diuretic

Source: UK Cropnet (http://ukcrop.net/perl/ace/grep/EthnobotDB).

FUNCTIONAL FOODS

Major edible legumes serving as functional foods include kidney bean (*Phaseolus vulgaris* L.), garden pea (*Pisum sativum* L.), and peanut (*Arachis hypogaea* L.). Kidney bean and garden pea contain the antifungal phytochemicals phaseolus β-1,3-glucanase and pisum sativum β-1,3 glucanase, respectively. Peanut seed contains two antioxidants, protocatechuic acid and lecithin. Peanut plants also contain esculetin, which has been shown to be anti-inflammatory and cancer preventive. Peanut seed contains lecithin (ranging from 5 to 7 mg per g), a commercially available antioxidant, which shows some anti-Alzheimer's disease activity and which may be effective for treating gall bladder disease.

Some minor food legumes are potentially of great importance as functional foods. Lablab [*Lablab purpureus* (L.) Sweet], also known as hyacinth bean, contains kievitone, which has antibacterial and antifungal activities as well as inhibiting breast cancer cell proliferation (Polya, 2003). Seeds from guar (*Cyamopsis tetragonolobus*) contain variable amounts of various flavones as well as isoflavones, plus substantial quantities of dietary fiber (Kays et al., 2006). Several products derived from guar, fenugreek, and red clover are commercially available. These products include Benefiber, a guar and wheat seed product used as a fiber supplement. In addition, several flavonoids and isoflavones are derived from both fenugreek and red clover.

Another legume, winged bean [*Psophocarpus tetragonolobus* (L.) DC], contains numerous phytochemicals useful as functional foods. Betulinic acid is not only apoptotic against cancer but it has antimelanoma activity as well. Winged bean contains tocopherol-rich oil, which improves utilization of vitamin A in the human body (Morris, 2004). The protein lectin derived from winged bean seeds is used in medical diagnostics, while erucic acid, also from winged bean, has antitumor potential. Additional chemicals from winged bean include beta-sitosterol, which is believed to be effective in treating benign prostatic hyperplasia and lowers LDL cholesterol levels. The flavonoids quercetin and delphinidin found in winged bean have antioxidant potential. Protocatechuic acid, betulinic acid, erucic acid, beta sitosterol, and quercetin are all commercially available.

Grass species that could be model functional foods include wheat, rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), corn, and oats (*Avena sativa* L.) (Polya, 2003). Diazepam from germinating wheat seed has been shown to be useful in skeletal muscle relaxation and can also be used as a tranquilizer. Bran and phytic acid from wheat are antioxidants, while bran is also a free radical scavenger. The barley phytochemical gramine is a free radical scavenger and antioxidant and also has antifungal properties. Corn and oats contain the antifungal chemicals *Zea mays* β-1,3-glucanase and avenacin A-1, A-2, B-1, B-2, respectively (Polya, 2003). Diazepam, phytic acid, and gramine are commercially available.

PHYTOCHEMICALS WITH MEDICINAL USES

Some legumes produce phytochemicals that can be used as medicines. These include leadtree [*Leucaena leucocephala* (Lam.) de Wit], *Canavalia brasiliensis* Mart. ex Benth., *Senna obtusifolia* (L.) H.S. Irwin & Barneby, *Desmodium heterophyllum* (Willd.) DC, and *D. uncinatum* (Jacq.) DC. Leaves and seeds from leadtree produce mimosine, which is responsible for free radical formation (Polya, 2003). *Canavalia brasiliensis* produces a lectin with apoptotic potential, while both *D. heterophyllum* and *D. uncinatum* seed produce soyasaponin αg, known to be an antioxidant and to scavenge free radicals. Interestingly, *S. obtusifolia* is a source of xylose, a caloric sugar that has been noted for possible diabetic applications. Mimosine and xylose (used as an ingredient) are both commercially available.

Some grass species with potential in the medicinal market include annual (Italian) ryegrass (*Lolium multiflorum* Lam.), lemon grass (*Andropogon citratus* DC), and crabgrass [*Digitaria exilis* (Kippist) Stapf]. Annual ryegrass is a source of 1-monolinolenin, which has been observed to be apoptotic against cancer cells. Lemon grass and close relatives produce geraniol, which has antiseptic properties. Crabgrass seed contains both luteolin and apigenin, which act as antibacterial agents. Because several crabgrass species are prolific perennial weeds in the southern United States, its periodic removal could be followed up with extractions for commercial luteolin and apigenin. Of the compounds indicated above, xylose and geraniol (used as an ingredient), as well as luteolin and apigenin are commercially available, suggesting an existence of a market for such compounds..

ENZYMES

The ability of plants to produce biomass from sunlight, water, carbon dioxide, and a few other nutrients, as well as an established infrastructure for harvesting and storage, has fostered the idea of using transgenic plants as "factories" for synthesizing such products as enzymes and plastics. Genetic transformation of alfalfa has resulted in plants capable of producing several enzymes of fungal origin, including phytase, α-amylase, and lignin peroxidase (LiP). Phytase is of particular interest owing to its importance in livestock feeding. Phytase breaks down phytic acid, a common

IMPLICATIONS FOR THE FUTURE: FLEXIBLE USE OF GRASSES AND LEGUMES

Butterfly pea (*Clitoria ternatea*). (Photo by J. Bradley Morris)

Commercial uses for legumes and grasses are emerging in a way that gives the grower flexibility in how and when these crops are used. The application of wet or dry fractionation technology is noted elsewhere in this chapter, but such large-scale processes may not be required (or even be a practical way) to add value to some grasses and legumes. For example, butterfly pea could be grown and marketed as an ornamental plant because of its beautiful flowers that range from white to dark blue. These same flowers, rich in anthocyanins, are also edible (appearing in salads at the Ritz Carlton in South Beach, FL) and are a potential source of nutraceuticals.

Several legumes that are useful as forages may at the same time be direct sources of nutraceuticals for livestock (i.e., no extraction needed). Hyacinth bean, for example, can be grown as a legume for pasture or hay, not only to provide energy and protein but also to take advantage of the nutraceutical properties from one of its components, the antimicrobial trigonelline, Year-end harvest of seed in ungrazed or recovering stands could provide an additional type of livestock feed supplement, while the forage itself could still be used as animal feed.

form of organic phosphorus in grain, releasing phosphate. Including phytase in the diets of swine and chickens, which do not produce this enzyme, improves utilization and minimizes excretion of phosphorus, the main agent of eutrophication of lakes and rivers. Phytase-containing transgenic alfalfa displays good agronomic performance (Ullah et al., 2002) and when fed to chickens, has been shown to reduce fecal phosphorus excretion.

Transgenic alfalfa containing a bacterial (*Bacillus licheniformis*) gene for α-amylase also displays good agronomic performance. Levels of expression at this point do not threaten industrial production of bacterial amylases (used in huge quantities in both grain ethanol and corn syrup industries). By contrast, transgenic alfalfa containing Mn-dependent LiP has shown somewhat less promise due to poor agronomic performance (Austin et al., 1995). The intended use for LiP—removal of lignin, an industrially undesirable component of wood and pulp effluent—is complicated by the fact that lignin is an important component of the plant cell. The negative growth effects of expressing the LiP genes in transgenic alfalfa may be due to the importance of lignin in plant structure, combined with improper compartmentalization of the enzymes within the transgenic plant.

Use of plants as enzyme factories has not yet fulfilled the vision of its proponents, but as techniques for improving genetic regulation in plants develop further, it is likely that some enzymes will be able to be produced in plants in sufficient quantities for industrial exploitation, provided that natural barriers to overexpression and localization in plant tissues can be overcome.

PLASTICS

Alfalfa has also been engineered to produce poly-β-hydroxybutyrate (PHB) and other polyhydroxyalkanoates that are used as biodegradable plastics. Insertion of genes from the bacterium *Ralstonia eutropha* has resulted in accumulation of granules of PHB in plant chloroplasts in amounts of 0.025 to 1.8 g per kg of plant dry weight (Saruul et al., 2002). At these modest concentrations, the transformed plants grew as well as the untransformed parents. While improving PHB yield without in turn negatively affecting plant growth would push this process toward industrial feasibility, transgenic alfalfa is up against the native *Ralstonia* bacterium itself, which has been shown in laboratory fermentations to accumulate up to 90% of its dry weight as PHB and thus is presently used for commercial PHB production.

FERMENTATIONS OF GRASS AND LEGUME BIOMASS TO COMMODITY CHEMICALS

The high carbohydrate content of grasses and legumes make them potential feedstocks for microbial fermentations to yield a host of products. Of these, ethanol has achieved the most attention, and indeed, several forages, particularly switchgrass (*Panicum virgatum* L.) have been touted as the likely basis for a cellulosic biomass industry. The role of grasses and legumes in biofuels is discussed in detail in Chapter 12 (Casler et al., 2009, this volume).

Fermentations of cellulosic biomass to chemical products can proceed via two different platforms (Fig. 13–1). The more intensively studied of these is the so-called sugar platform, in which biomass, pretreated to render the cellulose fraction more accessible, is combined with cellulase and other enzymes to saccharify (release sugars from) the biomass. The sugars are then fermented to the desired product, typically under anaerobic conditions, by specialist yeast or bacteria. The enzymes are produced in a separate batch fermentation of fungi (e.g., *Trichoderma reesei*) under aerobic conditions. An alternative approach, called consolidated bioprocessing (CBP), uses bacteria that produce their own cellulase and hemicellulase enzymes and subsequently ferment the products of enzymatic hydrolysis to various end products, primarily ethanol and organic acids (acetic, lactic, and succinic acids). As the name implies, CBP can be accomplished within a single reactor vessel. In principle, both platforms can be extended for the production of a variety of other chemicals (e.g., acetone, butanol, acetic acid, lactic acid, or succinic acid), although the potential number of products is more limited because known, wild-type CBP organisms are phylogenetically rather similar and have relatively similar metabolic characteristics. One product of CBP fermentations is the fermentation residue itself, which contains bacterial cells attached to the residual fiber via a sticky exopolysaccharide (EPS). This substance is produced by the bacteria themselves and enhances contact with the substrate to facilitate fiber degradation. The EPSs of several CBP bacteria have been characterized and differ from

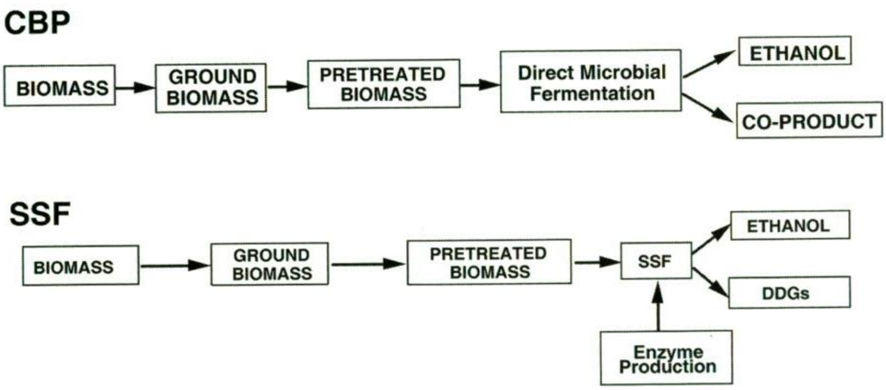


Fig. 13–1. Comparison of consolidated bioprocessing (CBP) with the conventional simultaneous saccharification and fermentation (SSF) platforms for producing ethanol from cellulosic biomass. Both platforms can be extended, by selection of proper fermentative organisms, to produce bulk chemical products other than ethanol. Potential coproducts of CBP include a fermentation residue with adhesive properties. DDG = distillers dried grain.

SOME TERMS DEFINED

Apoptotic—programmed cell death, or the facilitation of genetically determined destruction of cells (usually in reference to cancer cells) from within due to activation of a stimulus or removal of a suppressing agent or stimulus that is postulated to exist to explain the orderly elimination of superfluous cells.

Functional food—any food capable of providing additional benefits above and beyond human sustenance, including disease prevention benefits or energy after ingestion.

Medicinal food—any food having health-enhancing qualities and sometimes prescribed by medical doctors to provide health preventive care to human beings.

Nutraceutical—a blend of the words *nutrition* and *pharmaceutical*. The general category includes processed food made from functional food ingredients or fortified with health-promoting additives, like vitamin-enriched products, and also fresh foods (e g., vegetables) that have specific claims attached. Dietary supplements are nutraceuticals as well.

known microbial EPS in that they are rich in both xylose and mannose. While the EPS cannot be removed without destroying it, the entire fermentation residue has sufficient adhesive properties to permit bonding of wood panels. Like most other bio-based adhesives, the fermentation residue loses bond strength under wet conditions. Wet strength can be improved considerably by combining the residue with more water-resistant adhesives such as phenol-formaldehyde (PF) (Weimer et al., 2005). Use of the fermentation residue as a coadhesive provides a potential opportunity to replace a substantial fraction of the 1 billion pounds (450 million kg) of fossil fuel–derived PF used annually in the United States.

CONCLUSIONS

Although forages are prized for their ability to provide nutrients and fiber to both wild animals and livestock, we are beginning to realize their utility in a variety of other applications from structural materials to feedstocks for industrial fermentations to a source of fine chemicals. The growing awareness among consumers of natural foods and medicines and other types of natural products strongly suggests that further examination of forages as sources of these compounds is warranted. Taking advantage of commercial opportunities to bring these materials to the consumer will require marketing skill and a realistic assessment of the limited volumes of some of these markets. However, creative use of forages for applications beyond conventional animal feeding may ultimately tip the balance in favor of forage-based agriculture, with its attendant benefits to the environment and to sustainable development.

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